

(19) 日本国特許庁 (J P)

(12) 公表特許公報 (A)

(11) 特許出願公表番号

特表2003-518151

(P2003-518151A)

(43) 公表日 平成15年6月3日 (2003.6.3)

(51) Int.Cl. ⁷	識別記号	F I	テマコード* (参考)
C 0 8 G 65/325		C 0 8 G 65/325	4 B 0 5 0
C 0 8 F 8/30		C 0 8 F 8/30	4 J 0 0 5
C 1 2 N 9/96		C 1 2 N 9/96	4 J 1 0 0

審査請求 未請求 予備審査請求 有 (全 37 頁)

(21) 出願番号 特願2001-546734(P2001-546734)
 (86) (22) 出願日 平成12年12月18日 (2000. 12. 18)
 (85) 翻訳文提出日 平成14年6月20日 (2002. 6. 20)
 (86) 国際出願番号 P C T / U S 0 0 / 3 4 5 9 0
 (87) 国際公開番号 W O 0 1 / 0 4 5 7 9 6
 (87) 国際公開日 平成13年6月28日 (2001. 6. 28)
 (31) 優先権主張番号 6 0 / 1 7 1 , 8 3 4
 (32) 優先日 平成11年12月22日 (1999. 12. 22)
 (33) 優先権主張国 米国 (U S)

(71) 出願人 シアウォーター・コーポレーション
 アメリカ合衆国アラバマ州35806-2902,
 ハンツヴィル, ディスカヴァリー・ドライ
 ブ 490
 (72) 発明者 コズロウスキ, アントニ
 アメリカ合衆国 アラバマ 35816, ハ
 ンツヴィル, トランス ドライブ 4433
 (74) 代理人 弁理士 山本 秀策 (外2名)

最終頁に続く

(54) 【発明の名称】 ポリ (エチレングリコール) の1-ベンゾトリアゾリル炭酸エステルの調製のための方法

(57) 【要約】

本発明は、アミン塩基および有機溶媒の存在下で、水溶性の非ペプチド重合体の末端ヒドロキシ基を、炭酸ジ (1-ベンゾトリアゾリル) と反応させることによって水溶性の非ペプチド重合体の1-ベンゾトリアゾリル炭酸エステルを調製するための方法を提供する。この重合体の骨格は、ポリ (エチルグリコール) であり得る。次いで、この1-ベンゾトリアゾリル炭酸エステルは、生物学的に活性な因子と直接的に反応させて、生物学的に活性な重合体結合体を形成し得るか、またはアミノ酸 (例えば、リジン) と反応させて、アミノ酸誘導体を形成し得る。

[\[Sign In / Register \]](#)[Search](#) [Publish](#) [Resources](#) [Research and Analytics](#) [Blog](#) [About Us](#) [Contact Us](#)[Search Patents and Non-Patent Literature](#)

Entry for Patent Application Publication JP 2003-518151 A (03-Jun-2003)

[Front Page](#)[First Page Image](#)[Description](#)[Claims](#)

Japanese Application Publication (Source: DOCDB)

Publication No. JP 2003-518151 A published on 03-Jun-2003

Application No. JP 2001-546734 T filed on 18-Dec-2000

Priority

US 171834 P 22-Dec-1999 [-1]

Classifications

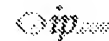
International (2006.01): C12N 9/96; A61K 47/48; C08F 8/30; C08G 65/325

International: C08G 65/325, 7C 8F, 7C 12N

European: A61K 47/48H4P; A61K 47/48W2

Also Published As

CA 2393638 A1	application	Method for the preparation of 1-benzotriazolyl carbonate esters of poly(Ethylene glycol)
EP 1259583 A2	application	Method for the preparation of 1-benzotriazolyl carbonate esters of water soluble polymers.
EP 2070968 A2	application	Method for the Preparation of 1-Benzotriazolyl Carbonate Esters of Poly(ethylene glycol)
US 2006/0264578	application	Method for the preparation of 1-benzotriazolyl carbonate esters of poly(ethylene glycol)
CA 2393638 C	patent	Method for the preparation of 1-benzotriazolyl carbonate esters of poly(Ethylene glycol)
EP 1259583 B1	patent	Method for the preparation of 1-benzotriazolyl carbonate esters of water soluble polymers.
US 7378469	patent	Method for the preparation of 1-benzotriazolyl carbonate esters of poly(ethylene glycol)



There's no image data available for this publication

[Options](#)[Download](#)[JP 2003-518150 A <](#)[JP 2003-518152 A >](#)[Terms of Use](#) | [Privacy Policy](#) | [Contact Us](#) |

Copyright © 2009-2011 IP.com. All Rights Reserved.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 June 2001 (28.06.2001)

PCT

(10) International Publication Number
WO 01/45796 A2

(51) International Patent Classification⁷: **A61P**

(21) International Application Number: PCT/US00/34590

(22) International Filing Date:
18 December 2000 (18.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/171,834 22 December 1999 (22.12.1999) US

(71) Applicant (for all designated States except US): **SHEAR-
WATER CORPORATION** [US/US]; 1112 Church Street,
Huntsville, AL 35801 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **KOZLOWSKI, An-
toni** [US/US]; 4433 Torrance Drive, Huntsville, AL 35816
(US).

(74) Agents: **HUMPHREY, Christopher, M.** et al.; Alston &
Bird LLP, P.O. Drawer 34009, Charlotte, NC 28234-4009
(US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AT
(utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA,
CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility
model), DK, DK (utility model), DM, DZ, EE, EE (utility
model), ES, FI, FI (utility model), GB, GD, GE, GH, GM,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK
(utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE PREPARATION OF 1-BENZOTRIAZOLYL CARBONATE ESTERS OF POLY(ETHYLENE GLYCOL)

(57) Abstract: The invention provides a method for preparing a 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer by reacting a terminal hydroxyl group of a water-soluble and non-peptidic polymer with di(1-benzotriazolyl)carbonate in the presence of an amine base and an organic solvent. The polymer backbone can be poly(ethylene glycol). The 1-benzotriazolylcarbonate ester can then be reacted directly with a biologically active agent to form a biologically active polymer conjugate or reacted with an amino acid such as lysine, to form an amino acid derivative.



WO 01/45796 A2

METHOD FOR THE PREPARATION OF 1-BENZOTRIAZOLYL CARBONATE ESTERS OF POLY(ETHYLENE GLYCOL)

FIELD OF THE INVENTION

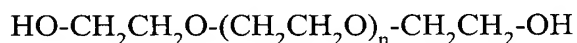
This invention relates to activated poly(ethylene glycol) derivatives and methods of preparing such derivatives.

5

BACKGROUND OF THE INVENTION

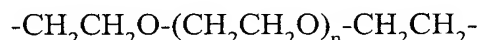
Covalent attachment of the hydrophilic polymer poly(ethylene glycol), abbreviated PEG, also known as poly(ethylene oxide), abbreviated PEO, to molecules and surfaces is of considerable utility in biotechnology and medicine. In its most common form, PEG is a linear polymer terminated at each end with hydroxyl groups:

10



The above polymer, alpha-,omega-dihydroxypoly(ethylene glycol), can be represented in brief form as HO-PEG-OH where it is understood that the -PEG- symbol represents the following structural unit:

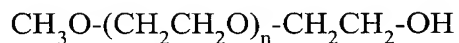
15



where n typically ranges from about 3 to about 4000.

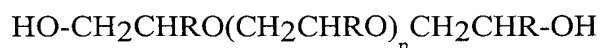
PEG is commonly used as methoxy-PEG-OH, or mPEG in brief, in which one terminus is the relatively inert methoxy group, while the other terminus is a hydroxyl group that is subject to ready chemical modification. The structure of mPEG is given below.

20



Random or block copolymers of ethylene oxide and propylene oxide, shown below, are closely related to PEG in their chemistry, and they can be substituted for PEG in many of its applications.

25



wherein each R is independently H or CH₃.

PEG is a polymer having the properties of solubility in water and in many organic solvents, lack of toxicity, and lack of immunogenicity. One use of PEG is to covalently attach the polymer to insoluble molecules to make the resulting PEG-molecule "conjugate" soluble. For example, it has been shown that the water-insoluble drug paclitaxel, when coupled to PEG, becomes water-soluble. Greenwald,
5 *et al.*, *J. Org. Chem.*, 60:331-336 (1995).

To couple PEG to a molecule, such as a protein, it is often necessary to "activate" the PEG by preparing a derivative of the PEG having a functional group at a terminus thereof. The functional group can react with certain moieties on the
10 protein, such as an amino group, thus forming a PEG-protein conjugate.

In U.S. Patent No. 5,650,234, which is incorporated by reference herein in its entirety, a 1-benzotriazolylcarbonate ester of poly(ethylene glycol) is described. The multi-step process described in the '234 patent for forming the 1-benzotriazolylcarbonate ester of PEG includes reaction of a PEG molecule with the
15 volatile and hazardous compound, phosgene, in order to form a PEG chloroformate intermediate. The use of phosgene in the process results in the formation of HCl, which can cause degradation of the PEG backbone. Due to the volatile nature of phosgene, and the resulting safety and quality problems associated with its use, there is a need in the art for a method for preparing 1-benzotriazolylcarbonate esters of PEG
20 without using phosgene.

SUMMARY OF THE INVENTION

The invention provides a method for the preparation of a 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer by reacting
25 the polymer with di(1-benzotriazolyl)carbonate. Using the invention, the 1-benzotriazolylcarbonate ester can be formed in a single step and without using phosgene, thereby avoiding the safety and quality problems associated with that compound.

30 The method of the invention includes providing a water-soluble and non-peptidic polymer having at least one terminal hydroxyl group and reacting the terminal hydroxyl group of the water-soluble and non-peptidic polymer with di(1-benzotriazolyl)carbonate to form the 1-benzotriazolylcarbonate ester of the water-

soluble and non-peptidic polymer. Examples of suitable water-soluble and non-peptidic polymers include poly(alkylene glycol), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxypropylmethacrylamide), poly(α -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), and copolymers, terpolymers, and mixtures thereof. In one embodiment, the polymer is poly(ethylene glycol) having an average molecular weight from about 200 Da to about 100,000 Da.

The reaction step can be conducted in the presence of an organic solvent and a base. Examples of suitable organic solvents include methylene chloride, chloroform, acetonitrile, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, and mixtures thereof. The base can be, for example, pyridine, dimethylaminopyridine, quinoline, trialkylamines, and mixtures thereof.

The method of the invention can further include reacting the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer with the amino groups of a second polymer having a plurality of primary amino groups, such as a protein, poly(ethylene glycol), aminocarbohydrates, or poly(vinylamine), to form a cross-linked polymer. Additionally, the 1-benzotriazolylcarbonate ester can be reacted with either an amino acid, such as lysine, to form a polymeric amino acid derivative, or a biologically active agent to form a biologically active polymer conjugate.

DETAILED DESCRIPTION OF THE INVENTION

The terms “functional group”, “active moiety”, “activating group”, “reactive site”, “chemically reactive group” and “chemically reactive moiety” are used in the art and herein to refer to distinct, definable portions or units of a molecule. The terms are somewhat synonymous in the chemical arts and are used herein to indicate that the portions of molecules that perform some function or activity and are reactive with other molecules. The term “active,” when used in conjunction with functional groups, is intended to include those functional groups that react readily with electrophilic or nucleophilic groups on other molecules, in contrast to those groups that require strong catalysts or highly impractical reaction conditions in order to react. For example, as would be understood in the art, the term “active ester” would include those esters that

react readily with nucleophilic groups such as amines. Typically, an active ester will react with an amine in aqueous medium in a matter of minutes, whereas certain esters, such as methyl or ethyl esters, require a strong catalyst in order to react with a nucleophilic group.

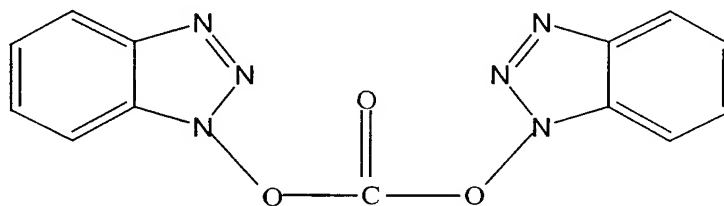
5 The term “linkage” or “linker” is used herein to refer to groups or bonds that normally are formed as the result of a chemical reaction and typically are covalent linkages. Hydrolytically stable linkages means that the linkages are substantially stable in water and do not react with water at useful pHs, e.g., under physiological conditions for an extended period of time, perhaps even indefinitely. Hydrolytically
10 unstable or degradable linkages means that the linkages are degradable in water or in aqueous solutions, including for example, blood. Enzymatically unstable or degradable linkages means that the linkage can be degraded by one or more enzymes. As understood in the art, PEG and related polymers may include degradable linkages in the polymer backbone or in the linker group between the polymer backbone and
15 one or more of the terminal functional groups of the polymer molecule.

 The term “biologically active molecule”, “biologically active moiety” or “biologically active agent” when used herein means any substance which can affect any physical or biochemical properties of a biological organism, including but not limited to viruses, bacteria, fungi, plants, animals, and humans. In particular, as used
20 herein, biologically active molecules include any substance intended for diagnosis, cure mitigation, treatment, or prevention of disease in humans or other animals, or to otherwise enhance physical or mental well-being of humans or animals. Examples of biologically active molecules include, but are not limited to, peptides, proteins, enzymes, small molecule drugs, dyes, lipids, nucleosides, oligonucleotides, cells,
25 viruses, liposomes, microparticles and micelles. Classes of biologically active agents that are suitable for use with the invention include, but are not limited to, antibiotics, fungicides, anti-viral agents, anti-inflammatory agents, anti-tumor agents, cardiovascular agents, anti-anxiety agents, hormones, growth factors, steroidal agents, and the like.

30 The invention provides a method for the preparation of a 1-benzotriazolylcarbonate ester (also referred to as a BTC ester) of a water-soluble and non-peptidic polymer, wherein a terminal hydroxyl group of a water-soluble and non-

peptidic polymer is reacted with di(1-benzotriazolyl)carbonate, the structure of which is shown below, to form the 1-benzotriazolylcarbonate ester.

Di(1-benzotriazolyl)carbonate, which should not pose significant safety or handling problems as a reagent and should not cause degradation of the polymer backbone, can be purchased as a 70% (w/w) mixture with 1,1,2-trichloroethane from Fluka Chemical Corporation of Milwaukee, WI.



di(1-benzotriazolyl)carbonate (diBTC)

The polymer backbone of the water-soluble and non-peptidic polymer can be poly(ethylene glycol) (i.e. PEG). However, it should be understood that other related polymers are also suitable for use in the practice of this invention and that the use of the term PEG or poly(ethylene glycol) is intended to be inclusive and not exclusive in this respect. The term PEG includes poly(ethylene glycol) in any of its forms, including alkoxy PEG, difunctional PEG, multiarmed PEG, forked PEG, branched PEG, pendent PEG (i.e. PEG or related polymers having one or more functional groups pendent to the polymer backbone), or PEG with degradable linkages therein.

PEG is typically clear, colorless, odorless, soluble in water, stable to heat, inert to many chemical agents, does not hydrolyze or deteriorate, and is generally non-toxic. Poly(ethylene glycol) is considered to be biocompatible, which is to say that PEG is capable of coexistence with living tissues or organisms without causing harm. More specifically, PEG is substantially non-immunogenic, which is to say that PEG does not tend to produce an immune response in the body. When attached to a molecule having some desirable function in the body, such as a biologically active agent, the PEG tends to mask the agent and can reduce or eliminate any immune response so that an organism can tolerate the presence of the agent. PEG conjugates tend not to produce a substantial immune response or cause clotting or other undesirable effects. PEG having the formula $-\text{CH}_2\text{CH}_2\text{O}-(\text{CH}_2\text{CH}_2\text{O})_n-\text{CH}_2\text{CH}_2-$, where n is from about 3 to about 4000, typically from about 3 to about 2000, is one

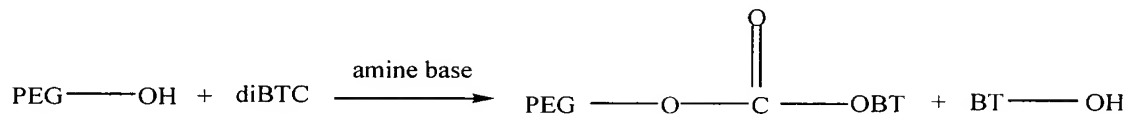
useful polymer in the practice of the invention. PEG having a molecular weight of from about 200 Da to about 100,000 Da are particularly useful as the polymer backbone.

5 The polymer backbone can be linear or branched. Branched polymer backbones are generally known in the art. Typically, a branched polymer has a central branch core moiety and a plurality of linear polymer chains linked to the central branch core. PEG is commonly used in branched forms that can be prepared by addition of ethylene oxide to various polyols, such as glycerol, pentaerythritol and sorbitol. The central branch moiety can also be derived from several amino acids,
10 such as lysine. The branched poly(ethylene glycol) can be represented in general form as $R(-\text{PEG}-\text{OH})_m$ in which R represents the core moiety, such as glycerol or pentaerythritol, and m represents the number of arms. Multi-armed PEG molecules, such as those described in U.S. Patent No. 5,932,462, which is incorporated by reference herein in its entirety, can also be used as the polymer backbone.

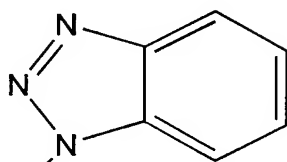
15 Many other polymers are also suitable for the invention. Polymer backbones that are non-peptidic and water-soluble, with from 2 to about 300 termini, are particularly useful in the invention. Examples of suitable polymers include, but are not limited to, other poly(alkylene glycols), such as poly(propylene glycol) ("PPG"), copolymers of ethylene glycol and propylene glycol and the like, poly(oxyethylated
20 polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxypropylmethacrylamide), poly(α -hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), such as described in U.S. Patent No. 5,629,384, which is incorporated by reference herein in its entirety, and copolymers, terpolymers, and mixtures thereof. Although the molecular weight
25 of each chain of the polymer backbone can vary, it is typically in the range of from about 100 Da to about 100,000 Da, often from about 6,000 Da to about 80,000 Da.

Those of ordinary skill in the art will recognize that the foregoing list for substantially water soluble and non-peptidic polymer backbones is by no means exhaustive and is merely illustrative, and that all polymeric materials having the
30 qualities described above are contemplated.

For purposes of illustration, a simplified reaction scheme for the method of the invention is shown below.



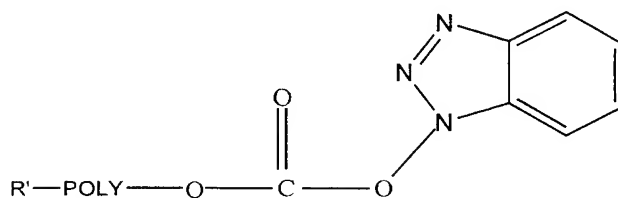
5



wherein BT is , L being the point of bonding to the oxygen atom.

In one embodiment, the reaction between the polymer and diBTC takes place in an organic solvent and in the presence of a base. Examples of suitable organic solvents include methylene chloride, chloroform, acetonitrile, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, and mixtures thereof. Amine bases, such as pyridine, dimethylaminopyridine, quinoline, trialkylamines, including triethylamine, and mixtures thereof, are examples of suitable bases. In one aspect of the invention, the molar ratio of di(1-benzotriazolyl) carbonate to the water-soluble and non-peptidic polymer is about 30:1 or less.

In one embodiment, the water-soluble and non-peptidic polymer has the structure R'-POLY-OH and the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer has the structure

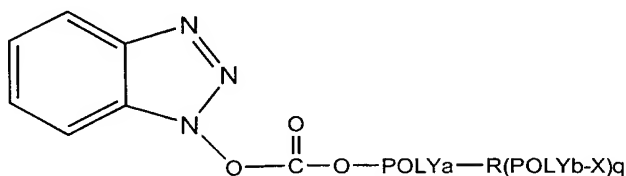


wherein POLY is a water-soluble and non-peptidic polymer backbone, such as PEG, and R' is a capping group. R' can be any suitable capping group known in the art for polymers of this type. For example, R' can be a relatively inert capping group,

such as an alkoxy group (e.g. methoxy). Alternatively, R' can be a functional group. Examples of suitable functional groups include hydroxyl, protected hydroxyl, active ester, such as N-hydroxysuccinimidyl esters and 1-benzotriazolyl esters, active carbonate, such as N-hydroxysuccinimidyl carbonates and 1-benzotriazolyl carbonates, acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, protected amine, protected hydrazide, thiol, protected thiol, carboxylic acid, protected carboxylic acid, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, and tresylate. The functional group is typically chosen for attachment to a functional group on a biologically active agent.

As would be understood in the art, the term "protected" refers to the presence of a protecting group or moiety that prevents reaction of the chemically reactive functional group under certain reaction conditions. The protecting group will vary depending on the type of chemically reactive group being protected. For example, if the chemically reactive group is an amine or a hydrazide, the protecting group can be selected from the group of tert-butyloxycarbonyl (t-Boc) and 9-fluorenylmethoxycarbonyl (Fmoc). If the chemically reactive group is a thiol, the protecting group can be orthopyridyldisulfide. If the chemically reactive group is a carboxylic acid, such as butanoic or propionic acid, or a hydroxyl group, the protecting group can be benzyl or an alkyl group such as methyl or ethyl. Other protecting groups known in the art may also be used in the invention.

In another embodiment, the water-soluble and non-peptidic polymer has the structure HO-POLYa-R(POLYb-X)_q and the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer has the structure



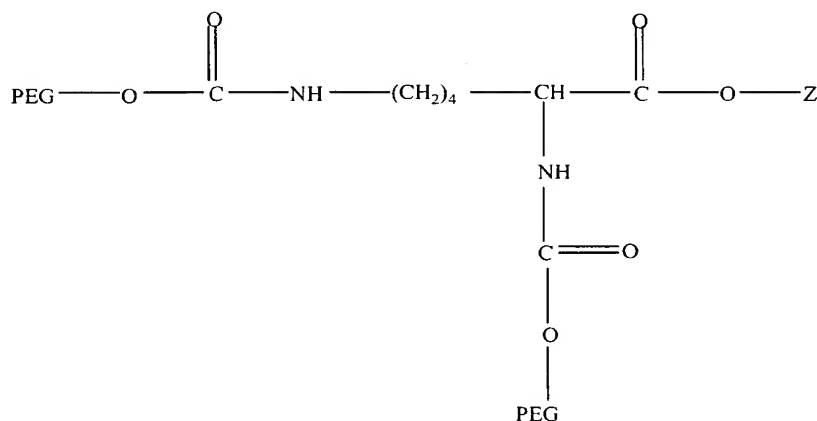
wherein POLYa and POLYb are water-soluble and non-peptidic polymer backbones, such as PEG, that may be the same or different;

R is a central core molecule, such as glycerol or pentaerythritol;
 q is an integer from 2 to about 300; and
 each X is a capping group.

The X capping groups may be the same as discussed above for R'.

5 In another aspect, a difunctional or higher functional BTC ester of the water-soluble and non-peptidic polymer is reacted with at least two amino groups of a second polymer having a plurality of primary amino groups, such as amino PEGs or other multifunctional amine polymers, such as proteins, aminocarbohydrates, or poly(vinylamine), to form cross-linked polymers. The amine polymer will generally
 10 have three or more available amino groups. Such polymers form hydrogels; that is, they become highly hydrated in aqueous media, but do not dissolve. Since these hydrogels are commonly biocompatible and may be degradable, many biomedical applications are possible in the areas of drug delivery, wound covering, and adhesion prevention.

15 A further embodiment of the invention involves the reaction of BTC esters of water-soluble and non-peptidic polymers with amino acids to form amino acid derivatives. In one embodiment, PEG-BTC esters are reacted with lysine to form a polymeric lysine derivative. For example, one such lysine derivative is a doubly PEGylated lysine, wherein the two PEGs are linked to the lysine amines by carbamate
 20 bonds, as shown below.



wherein PEG is poly(ethylene glycol) and Z is selected from the group consisting of H, N-succinimidyl, or 1-benzotriazolyl.

Such PEG derivatives of lysine are useful as reagents for preparation of PEG derivatives of proteins. These PEG derivatives often offer advantages over non-PEGylated proteins, such as longer circulating life-times *in vivo*, reduced rates of proteolysis, and lowered immunogenicity. In another aspect, PEG BTC derivatives are used directly in attaching PEG to proteins through carbamate linkages and may offer advantages similar to those described for the lysine PEG derivatives.

BTC esters of water-soluble and non-peptidic polymers can also be reacted with biologically active agents to form biologically active polymer conjugates. Examples of biologically active agents include peptides, proteins, enzymes, small molecule drugs, dyes, lipids, nucleosides, oligonucleotides, cells, viruses, liposomes, microparticles and micelles.

The invention also includes 1-benzotriazolylcarbonate esters of water-soluble and non-peptidic polymers prepared according to the above-described process. As noted above, it is believed that polymer derivatives prepared according to the invention exhibit higher quality because degradation of the polymer backbone caused by phosgene is avoided. Further, since the method of the invention requires only one step and fewer reactants, process efficiency is enhanced and cost is reduced.

The following examples are given to illustrate the invention, but should not be considered in limitation of the invention.

EXPERIMENTAL

Example 1

Preparation of mPEG₅₀₀₀BTC

A solution of mPEG₅₀₀₀-OH (MW 5000, 15 g, 0.003 moles), di(1-benzotriazolyl) carbonate (4.0 g of 70% mixture, 0.000945 moles), and pyridine (2.2 ml) in acetonitrile (30 ml) was stirred at room temperature under nitrogen overnight. The solvent was removed by distillation, the residue was dissolved in 80 ml of methylene chloride, and the resulting solution was added to 850 ml of ethyl ether. The mixture was cooled to 0-5°C and the precipitate was collected by filtration. The precipitation process was then repeated to obtain a white solid which was dried under vacuum at room temperature to yield 13.5 g of product which was shown by ¹H nmr to be 100% substituted. ¹H nmr (dmso d-6): 3.23 ppm, CH₃O; 3.51 ppm, O-CH₂CH₂-

O; 4.62 ppm, m, mPEG-O-CH₂-OCO₂- ; 7.41-8.21, complex mult., benzotriazole protons.

Example 2

5

Preparation of mPEG_{20,000}BTC

A solution of mPEG_{20,000}-OH (MW 20,000, 20 g, 0.001 moles), di(1-benzotriazolyl) carbonate (3.4 g of 70% mixture, 0.00803 moles), and pyridine (3.0 ml) in acetonitrile (40 ml) was stirred at room temperature under nitrogen overnight. The solvent was removed by distillation and the residue was dissolved in 80 ml of methylene chloride and the resulting solution was added to 800 ml of ethyl ether. The precipitate was collected by filtration and was dried under vacuum at room temperature to yield 16.8 g of product which was shown by ¹H nmr to be 100% substituted. ¹H nmr (dmso d-6): 3.23 ppm, CH₃O; 3.51 ppm, O-CH₂CH₂-O; 4.62 ppm, m, mPEG-O-CH₂-OCO₂- ; 7.41-8.21, complex mult., benzotriazole protons.

15

Example 3

Derivatization of lysine with mPEG_{20,000}BTC

Lysine.HCl (0.0275 g, 0.000151 moles) was dissolved in 26 ml of 0.1 M borate buffer and the pH was adjusted to 8.0 with 0.1 M NaOH. To the resulting solution was added mPEG_{20,000} BTC (7.0 g, 0.00350 moles) over 15 minutes and the pH was kept at 8 by addition of 0.1 M NaOH. After stirring the resulting solution for 3 h, 15 g of H₂O and 4 g of NaCl were added and the pH was adjusted to 3.0 with 10% phosphoric acid. The product was extracted with methylene chloride and the extract dried over MgSO₄. After concentrating the solution to 30 ml, the solution was poured into 300 ml of ethyl ether and the product collected by filtration and dried under vacuum at room temperature to yield 5.9 g of product as a white solid. Analysis by gel permeation chromatography (Ultrahydrogel 250, column temperature 75°C, aqueous buffer pH 7.2) showed the product to be a mixture of di-N-PEGylated lysine (MW ~ 40 KDa, 63.05%), mono-N-PEGylated lysine (MW ~ 20 KDa, 36.95%), and mPEG_{20,000}.

30

Example 4Derivatization of lysozyme with mPEG₅₀₀₀BTC

To 4 ml of lysozyme solution (3 mg/ml in 50 mM sodium phosphate buffer, pH 7.2) was added 20.3 mg of mPEG₅₀₀₀ BTC (5-fold excess of mPEG₅₀₀₀ BTC) and the mixture was continually mixed at room temperature. Analysis by capillary electrophoresis (57cm x 76um column; 30mM phosphate buffer; operating voltage 25 kV) after 4 hours showed that 6.94% of unreacted lysozyme remained, while 33.99 % of mono-PEGylated lysozyme, 43.11% di-PEGylated lysozyme, 13.03% tri-PEGylated lysozyme, and 2.92% of tetra-PEGylated lysozyme had formed.

Example 5PEG_{2KDa}- α -hydroxy- ω -propionic acid, benzyl ester

To a solution of PEG_{2KDa}- α -hydroxy- ω -propionic acid (10g, 0.0050 moles)(Shearwater Corp.) in anhydrous methylene chloride (100 ml) 1-hydroxybenzotriazole (0.30g), 4-(dimethylamino)pyridine (1.0g), benzyl alcohol (10.8g, 0.100 moles) and 1,3-dicyclohexylcarbodiimide (1.0 M solution in methylene chloride, 7.5 ml, 0.0075 moles) were added. The reaction mixture was stirred overnight at room temperature under argon. The mixture was then concentrated to about 50 ml, filtered and added to 800 ml cold diethyl ether. The precipitated product was filtered off and dried under reduced pressure. Yield 8.2g. NMR (d₆-DMSO): 2.60 ppm (t, -CH₂-COO-), 3.51 ppm (s, PEG backbone), 4.57 ppm (t, -OH-), 5.11 ppm (s, -CH₂- (benzyl)), 7.36 ppm (m, -C₆H₅ (benzyl)).

Example 6PEG_{2KDa}- α -benzotriazole carbonate- ω -propionic acid, benzyl ester

To a solution of PEG_{2KDa}- α -hydroxy- ω -propionic acid, benzyl ester (8.2g, 0.0025 moles) in acetonitrile (82 ml), pyridine (0.98 ml) and di(1-benzotriazolyl)carbonate (1.48g) were added and the reaction mixture was stirred overnight at room temperature under argon atmosphere. The mixture was then filtered and solvent was evaporated to dryness. The crude product was dissolved in methylene chloride and precipitated with isopropyl alcohol. The wet product was dried under reduced pressure. Yield 6.8g. NMR (d₆-DMSO): 2.60 ppm (t, -CH₂-COO-), 3.51 ppm (s, PEG backbone), 4.62 ppm (m, -CH₂-O(C=O)-), 5.11 ppm (s, -CH₂- (benzyl)), 7.36 ppm (m, -C₆H₅ (benzyl)), 7.60 – 8.50 ppm (4m, aromatic protons of benzotriazole).

THAT WHICH IS CLAIMED IS:

1. A method for the preparation of a 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer, comprising:

5 providing a water-soluble and non-peptidic polymer having at least one terminal hydroxyl group; and

reacting the terminal hydroxyl group of the water-soluble and non-peptidic polymer with di(1-benzotriazolyl)carbonate to form a 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer.

10

2. The method of Claim 1, wherein the water-soluble and non-peptidic polymer is selected from the group consisting of poly(alkylene glycol), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxypropylmethacrylamide), poly(α -hydroxy acid), poly(vinyl alcohol),
15 polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), and copolymers, terpolymers, and mixtures thereof.

3. The method of Claim 1, wherein the water-soluble and non-peptidic polymer is poly(ethylene glycol).

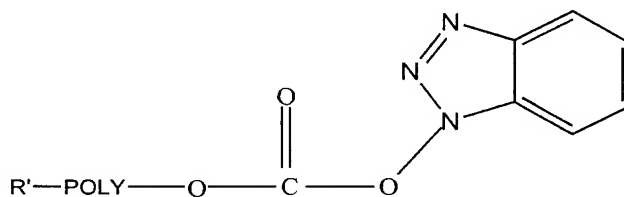
20

4. The method of Claim 3, wherein the poly(ethylene glycol) has an average molecular weight from about 200 Da to about 100,000 Da.

5. The method of Claim 1, wherein the water-soluble and non-peptidic polymer has from about 2 to about 300 termini.

25

6. The method of Claim 1, wherein the water-soluble and non-peptidic polymer has the structure R'-POLY-OH and the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer has the structure



wherein POLY is a water-soluble and non-peptidic polymer backbone and R' is a capping group.

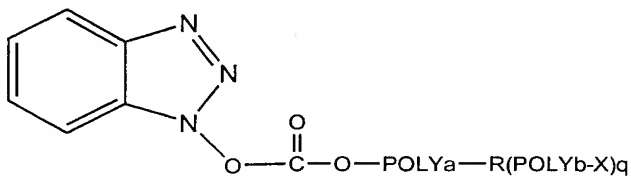
7. The method of Claim 6, wherein POLY is poly(ethylene glycol).

8. The method of Claim 7, wherein the poly(ethylene glycol) has an average molecular weight from about 200 Da to about 100,000 Da.

9. The method of Claim 6, wherein R' is methoxy.

10. The method of Claim 6, wherein R' is a functional group selected from the group consisting of hydroxyl, protected hydroxyl, active ester, active carbonate, acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, protected amine, protected hydrazide, thiol, protected thiol, carboxylic acid, protected carboxylic acid, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, and tresylate.

11. The method of Claim 1, wherein the water-soluble and non-peptidic polymer has the structure HO-POLYa-R(POLYb-X)_q and the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer has the



structure

5

wherein POLYa and POLYb are water-soluble and non-peptidic polymer backbones that may be the same or different;

R is a central core molecule;

q is an integer from 2 to about 300; and

10

each X is a capping group.

12. The method of Claim 11, wherein POLYa and POLYb are poly(ethylene glycol).

15

13. The method of Claim 12, wherein POLYa and POLYb each have an average molecular weight from about 200 Da to about 100,000 Da.

20

14. The method of Claim 11, wherein each X is independently selected from the group consisting of alkoxy, hydroxyl, protected hydroxyl, active ester, active carbonate, acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, protected amine, protected hydrazide, thiol, protected thiol, carboxylic acid, protected carboxylic acid, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, and tresylate.

25

15. The method of Claim 1, wherein said reacting step is conducted in an organic solvent.

16. The method of Claim 15, wherein the organic solvent is selected from the group consisting of methylene chloride, chloroform, acetonitrile, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, and mixtures thereof.

5 17. The method of Claim 1, wherein said reacting step is conducted in the presence of a base.

18. The method of Claim 17, wherein the base is selected from the group consisting of pyridine, dimethylaminopyridine, quinoline, trialkylamines, and
10 mixtures thereof.

19. The method of Claim 1, wherein the molar ratio of di(1-benzotriazolyl) carbonate to the water-soluble and non-peptidic polymer is about 30:1 or less.

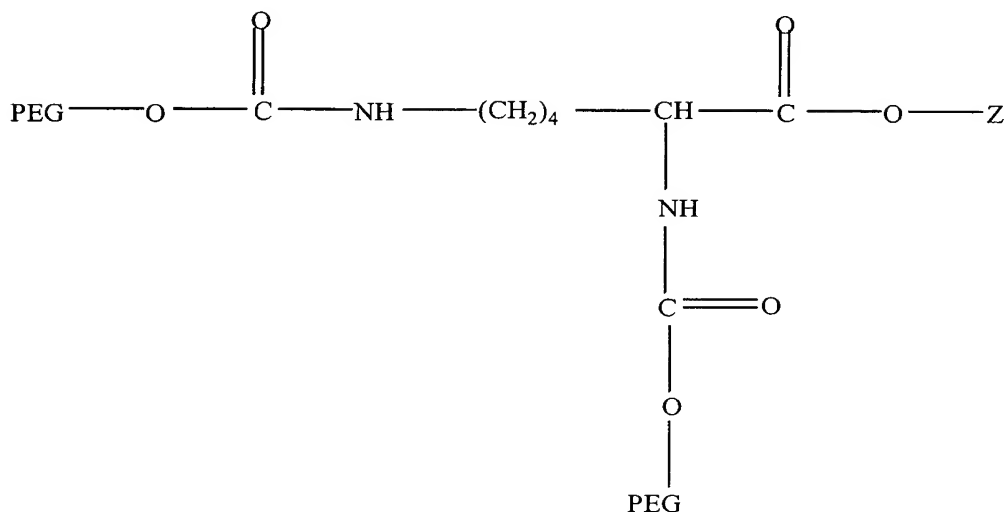
15 20. The method of Claim 1, further comprising the steps of:
 providing a second polymer having a plurality of primary amino groups; and
 reacting the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer with at least two of the amino groups of the second polymer to
20 form a cross-linked polymer.

21. The method of Claim 20, wherein the second polymer is selected from the group consisting of proteins, aminopoly(ethylene glycol), aminocarbohydrates, and poly(vinylamine).
25

22. The method of Claim 1, further comprising the step of reacting the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer with an amino acid to form an amino acid derivative.

30 23. The method of Claim 22, wherein the amino acid is lysine.

24. The method of Claim 23, wherein the amino acid derivative has the structure



wherein PEG is poly(ethylene glycol) and Z is selected from the group consisting of H, N-succinimidyl, or 1-benzotriazolyl.

25. The method of Claim 1, further comprising the step of reacting the 1-benzotriazolylcarbonate ester of the water-soluble and non-peptidic polymer with a biologically active agent to form a biologically active polymer conjugate.

26. The method of Claim 25, wherein the biologically active agent is selected from the group consisting of peptides, proteins, enzymes, small molecule drugs, dyes, lipids, nucleosides, oligonucleotides, cells, viruses, liposomes, microparticles and micelles.

27. A 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer prepared according to the process of Claim 1.

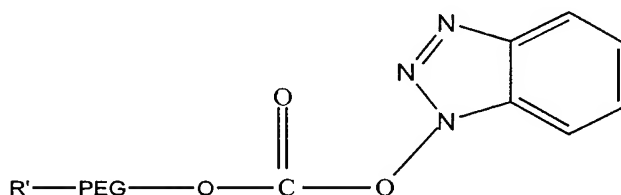
28. A method for the preparation of a 1-benzotriazolylcarbonate ester of a water-soluble and non-peptidic polymer, comprising:

providing a poly(ethylene glycol) molecule with a terminal hydroxyl group and an average molecular weight from about 200 Da to about 100,000 Da and having the structure



wherein R' is a capping group; and

reacting the terminal hydroxyl group with di(1-benzotriazolyl)carbonate to form a 1-benzotriazolylcarbonate ester of the poly(ethylene glycol) having the structure



wherein R' is as defined above.

29. The method of Claim 28, wherein R' is methoxy.

30. The method of Claim 28, wherein R' is a functional group selected from the group consisting of hydroxyl, protected hydroxyl, active ester, active carbonate, acetal, aldehyde, aldehyde hydrates, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, protected amine, protected hydrazide, thiol, protected thiol, carboxylic acid, protected carboxylic acid, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxals, diones, mesylates, tosylates, and tresylate.